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Research Note

A Simple Method for the Purification of Trichostrongyle Egg Shells

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ABSTRACT: Biochemical analysis of nematode egg shells requires the availability of pure material. Eggs of the ruminant nematode, *Haemonchus contortus,* were purified from sheep feces by flotation on sucrose and Percoll. Unembryonated eggs were disrupted by chopping and sonication, and pure shell preparations were recovered by differential centrifugation on Percoll gradients. For recovery of shells after hatching, eggs were allowed to embryonate, then shells and hatched larvae were separated on Percoll gradients.

KEY WORDS: Nematoda, *Haemonchus contortus,* egg shell, hatching.

The nematode egg shell serves as the primary barrier to adverse environmental conditions (Wharton, 1980). An in-depth knowledge of the composition of the egg shell would aid in understanding both how the shell functions in protecting the juvenile parasite and how it is degraded during the hatching process. However, studies on the nematode egg shell are limited by the lack of significant quantities of pure material. The inability to separate shell components from embryonic material makes biochemical analyses of shell components impractical. In order to study the composition of the egg shell of the ruminant trichostrongyle *Haemonchus contortus* (Rudolphi, 1803), it was necessary to develop methods for the recovery of large quantities of pure egg shells, both pre- and posthatching. Two methods are described here which should be applicable, with minor modifications, to egg shell recovery from other nematode species.

The Beltsville strain of *Haemonchus contortus* was maintained by serial passage in parasite-naive polled Dorset lambs (2–6 mo of age). For egg recovery, lambs were inoculated with 10,000–15,000 third-stage larvae obtained from 10-day fecal cultures. Egg production was monitored by flotation of fecal samples on 150% sucrose and eggs were enumerated by the modified McMaster technique (Whitlock, 1948). Fecal material was used for large scale egg recovery only when numbers of eggs per gram (EPG) of feces were $>10^3$. On average, 300 g of feces from 3 lambs shedding over 1,000 EPG was used to produce 0.5 ml of purified packed eggs and subsequently 50 μl of purified packed egg shells.

For egg recovery, feces from source lambs (300 g or 500 ml) were mixed with an equal volume of tap water, then homogenized briefly in a blender to create a slurry. Fecal material was then passed through a 300-μm mesh screen to remove large debris, and the screen rinsed with several volumes of tap water. The screened fecal suspension, containing eggs, was mixed with an equal volume of 150% sucrose, placed into 50-ml plastic tubes and centrifuged at a relative centrifugal force of 1,400 *g* in a Sorvall RC-3B centrifuge (Dupont Company, Wilmington, Delaware). Floated eggs were aspirated from the surface of the sucrose suspension, diluted in 100 volumes of tap water, and allowed to settle in a 2-liter beaker for 30 minutes. The supernatant was aspirated leaving settled eggs in 200 ml of water. This suspension was mixed with an equal volume of 150% sucrose and centrifuged as above. Following aspiration from the second sucrose flotation, eggs were pelleted, washed once in tap water by centrifugation (10 minutes at 1,400 *g*), and examined. Egg preparations from sucrose flotation contained varying amounts of fecal debris. For further purification, eggs were underlaid with Percoll (Sigma, St. Louis, Missouri) at concentrations of 30% and 45%, and centrifuged at 1,400 *g* for 15 min. Eggs recovered from the interface of the 30% and 45% Percoll layers were found to be free of fecal debris.

For the recovery of egg shells from unembryonated eggs, freshly recovered eggs were concentrated by centrifugation (1,400 *g*) to a small volume (1–2 ml) and chopped extensively with razor blades in a glass petri dish. Following chopping, shells were further disrupted by brief sonication (30% power for 1 min using a Microson Ultrasonic Cell Disrupter, Ultrasonic Heat Systems, Farmingdale, New York). This treatment was effective in releasing embryonic material without significant destruction of the shell. After sonication, 10–20 volumes of distilled water were

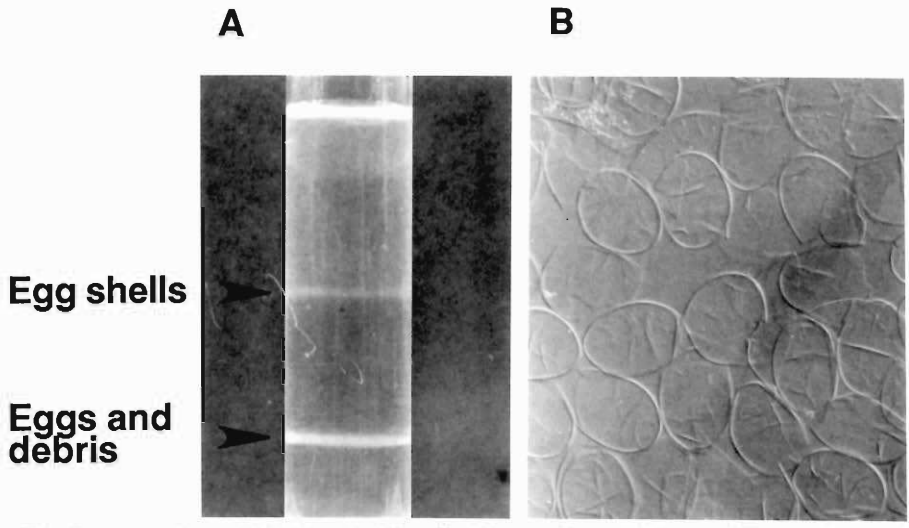

Figure 1. Recovery of egg shells from freshly collected, unembryonated eggs of *Haemonchus contortus*. A. Percoll gradient showing the separation of egg shells from undisrupted eggs and debris. B. Egg shells recovered from the Percoll gradient (×200).

added to dilute the preparation, then solubilized embryonic material was removed from shells and undisrupted eggs by centrifugation at 600 *g*. The supernatant, containing the majority of the embryonic debris, was aspirated and the pellet resuspended in 2–5 ml of distilled water. To recover shells from residual embryonic material and intact eggs, the chopped, sonicated preparations were underlaid with Percoll at various concentrations ranging from 10% to 50% and centrifuged at 600 *g* for 15 min. Based on recoveries from these gradients, 20% Percoll was found to be the most effective at separating shells from intact eggs. Using a 20% and 50% gradient, pure egg shells banded on top of the 20% Percoll, whereas debris, including intact eggs, was recovered from the interface of the 20% and 50% layers (Fig. 1). Egg shells did not appear to be substantially disrupted by this procedure and were free of embryonic debris when harvested from the gradients. Adequate separation of egg shells was achieved using a starting number of eggs ranging from 1.3 to 10.4 × 10^6 in a volume of 0.25–2.0 ml.

For recovery of egg shells following hatching, eggs were embryonated and hatched as follows. Eggs were purified as described above, then washed repeatedly in sterile tap water supplemented with 50 U/ml of penicillin, and 50 μg/ml of streptomycin. To assess sterility, a 50-μl sample of the final egg preparation was streaked onto nutrient agar plates prior to the initiation of hatching cultures. Preparations found to be contaminated with bacteria were discarded. Eggs, sterilized by antibiotic treatment, were incubated at 25°C for 42 hr in sterile tap water. After this time period, >90% of eggs had embryonated and larvae had hatched. For recovery of shells from hatched larvae and debris, cultures were concentrated to 2–5 ml by centrifugation (1,400 *g*), underlaid with an equal volume of various concentrations of Percoll ranging from 10% to 50% and centrifuged at 600 *g* for 15 min. Hatched egg shells were found to float on 10% Percoll, whereas debris floated on 35% Percoll and hatched larvae floated on 50% Percoll. Based on these results, a gradient was constructed consisting of 10%, 35%, and 50% Percoll. This gradient resulted in excellent separation of shells from debris, including unhatched eggs, and hatched larvae (Fig. 2). The egg shells recovered using this procedure were relatively intact. In addition, viable first-stage larvae could be recovered in large numbers. These larvae could be used further following active migration through 20-μm mesh nylon screens to recover viable larvae from dead or nonmotile worms.

Studies of nematode egg shells have been limited to morphological descriptions of the shell, histological staining, and, in a few cases, limited biochemical analyses (Clarke et al., 1967; Bird, 1971; Wharton, 1980, 1983). Further studies of

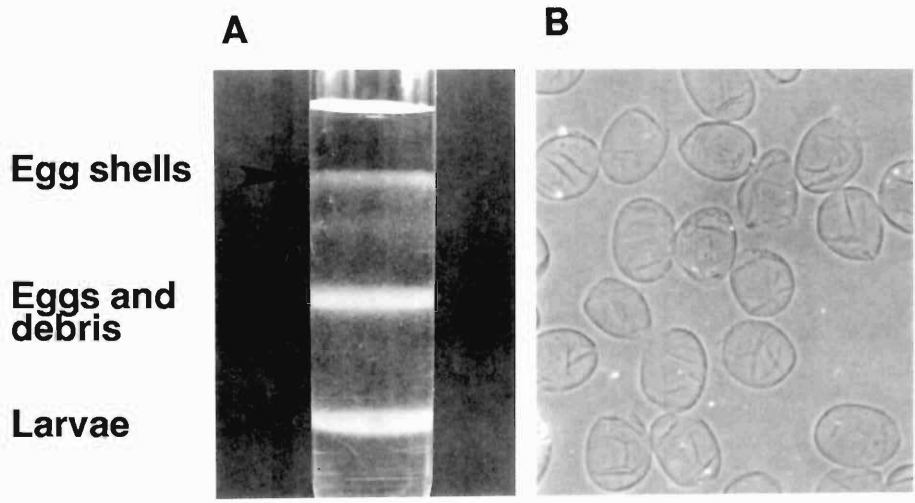

Figure 2. Recovery of egg shells from hatched *Haemonchus contortus* eggs. A. Percoll gradient showing the separation of egg shells, first-stage larvae, and unhatched eggs and debris. B. Egg shells recovered from the Percoll gradient (×200).

the nematode egg shell, its composition, formation, and degradation, have been limited by the availability of pure material. Using the procedures described here for obtaining pure egg shells, it should be possible to define, characterize, and separate the components of the egg shell of *Haemonchus contortus*. Additionally, purified egg shell components can be used to generate antibodies for localization of extracted components within the shell. Minor modifications of the described methods should make it feasible to recover significant amounts of pure egg shells from a variety of species of nematodes.

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